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PESTS NOT KNOWN TO OCCUR IN THE UNITED STATES OR OF LIMITED DISTRIBUTION NO. 91: *Phoma tracheiphila*

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Disease

MAL SECCO

Pathogen

Phoma tracheiphila (Petri) Kantschaveli & Gikachvili

Other Names

Deuterophoma tracheiphila Petri
Bakerophoma tracheiphila (Petri) Ciferri

Class: Order

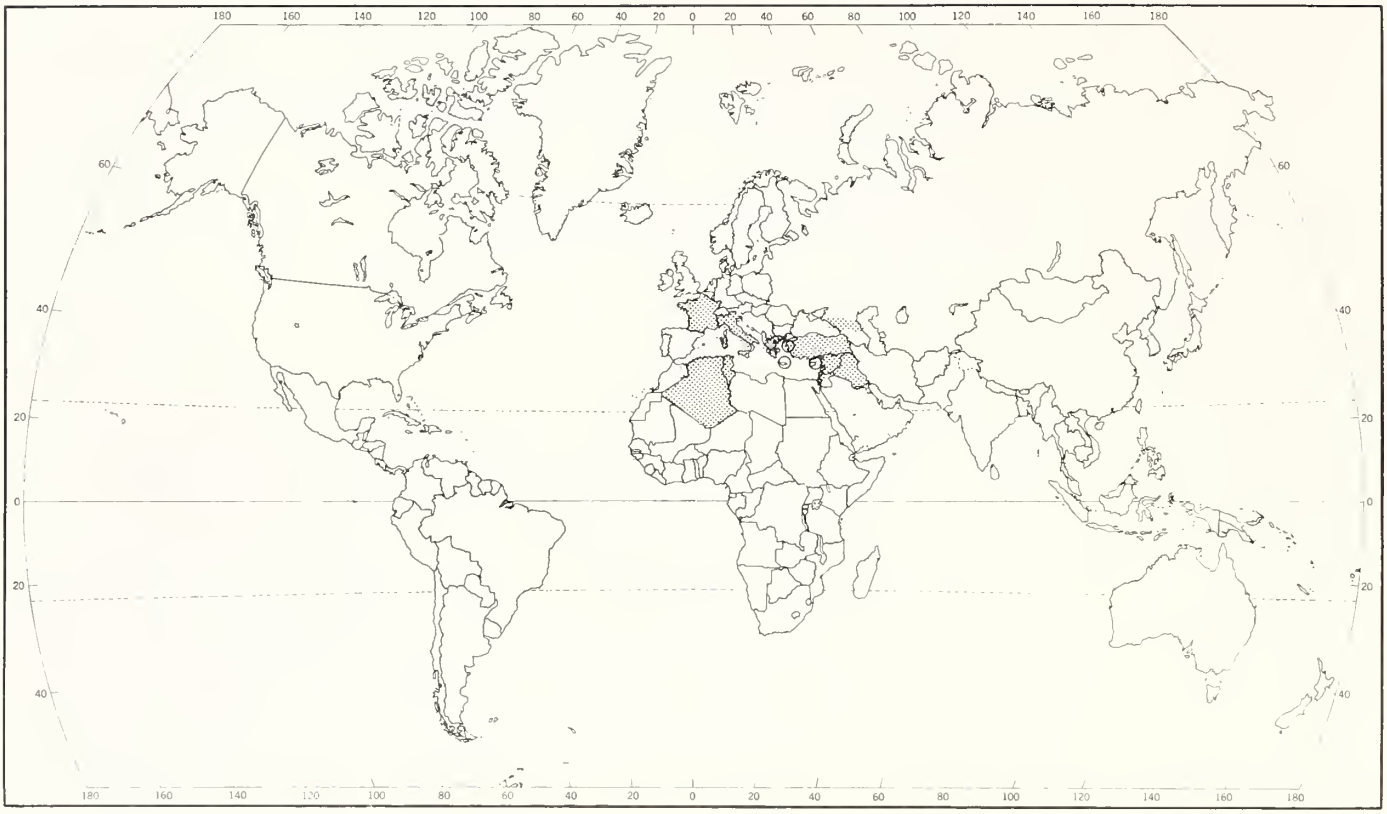
Coelomycetes: Sphaeropsidales

Economic Importance

Mal secco, caused by *Phoma tracheiphila*, is considered the most serious disease of Italian citriculture (Salerno and Cutuli 1976, 1979, 1981) and the most destructive fungal disease of lemon in the Mediterranean region (Graniti and Perrotta 1988). During 1931-51, lemon production in Italy decreased 50 percent due to mal secco (Burke 1951). Reduction in lemon yield in Italy has resulted in estimated annual losses of more than US$160 million. The fungus kills nearly one million trees yearly (Salerno and Cutuli 1979, 1981). Because the disease not only lowers production but also kills trees adds to the seriousness of the disease.

Hosts

Phoma tracheiphila has a wide range of natural hosts in the genus *Citrus*, as well as *Poncirus trifoliata*, *Fortunella* spp., and *Severinea buxifolia* (Catara and Cutuli 1975, Ciccarone and Russo 1983, Grasso 1984, Grasso and Perrotta 1978, Solel and Oren 1975), all members of the Rutaceae. The disease is particularly severe on *C. limon* (lemon), as well as *C. medica* (citron), and *C. deliciosa* (mandarin), including some of its hybrids (Punithalingam and Holliday 1973, Z. Solel, pers. comm.). *C. aurantiifolia* (lime) has been severely affected in Israel. *C. sinensis* (sweet orange) varies in susceptibility. *C.* x *paradisi* (grapefruit) and *C. reticulata* (tangerine) are rarely affected (Z. Solel, pers. comm.). Infection on rootstocks ranges from high severity on *C. jambhiri* (rough lemon) and *C. aurantium* subsp. *bergamia* (bergamot) to low severity on *C. reshni* (cleopatra mandarin), *C. volkameriana* (nom. illegit. (Volkamer citrus)), *Poncirus trifoliata*, (trifoliate orange), and *P. trifoliata* x *C. sinensis* (troyer citrange) (Punithalingam and Holliday 1973, Z. Solel, pers. comm.). *C. aurantium* (sour orange) is very susceptible in Italy, but only moderately affected in Israel (Salerno and Cutuli 1979, Z. Solel, pers. comm.). Almost all *Citrus* spp., their relatives, and hybrids are susceptible to artificial inoculation of *P. tracheiphila*.



Phoma tracheiphila distribution map (Prepared by Technical Information Systems Staff, PPQ, APHIS, USDA).

General Distribution

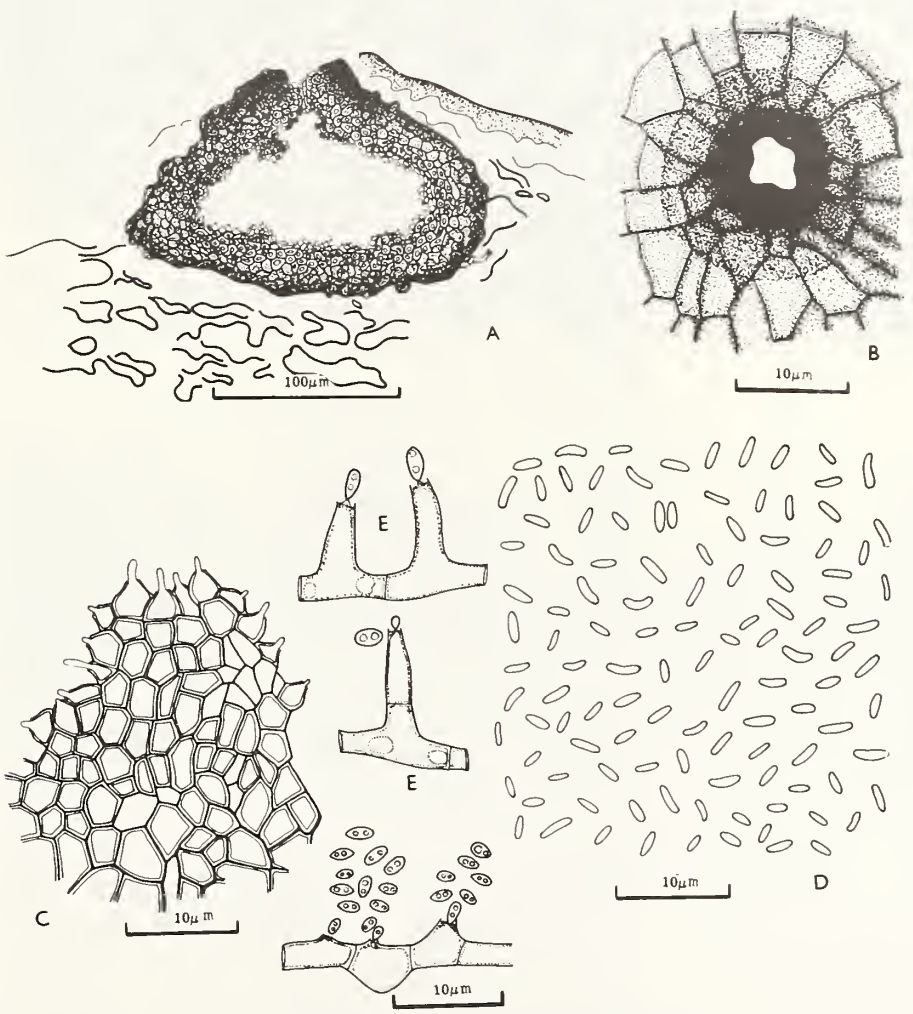
Mal secco occurs in the Mediterranean basin and the eastern coast of the Black Sea (Punithalingam and Holliday 1973); its distribution is limited by microclimate (J. Menge, pers. comm.). The Commonwealth Mycological Institute (1978) indicated that mal secco had been reported from the following countries: Aegean Islands, Algeria, Cyprus, France (Menton), Greece (including Crete), Iraq, Israel, Italy (including Sicily and Sardinia), Lebanon, the Soviet Union (Georgia and Caucasus), Syria, Tunisia, and Turkey. The report of mal secco in Spain (Laborda and Sanchez 1974) is apparently erroneous (A. Alfaro, pers. comm., Gimenez-Verdu 1982).

Characters

On stems, pycnidia immersed and aggregated in dead ashy gray areas of withered twigs, occasionally in cracks in bark, pruning cuts, or leaf scars (Graniti and Perrotta 1988, Holliday 1980, Petri 1930). Pycnidia globose to lenticular (Fig. 1A), black, ostiolate (Fig. 1B), 100-180 µm diameter, wall several cells thick: outer wall of sclerotized, brown to

black cells; inner wall of hyaline, thin-walled, pseudoparenchymatic cells (Fig. 1C). Conidiogenous cells enteroblastic, phialidic (Ciccarone and Russo 1969), hyaline, simple, ampuliliform (Fig. 1C), lining pycnidial cavity. Conidia (Fig. 1D) hyaline, unicellular, straight or curved, apices rounded, 2-3 x 1 µm (Punithalingam and Holliday 1973). Hyphal conidia (Fig. 1E) produced on exposed wood surfaces, wounded plant tissues, and within host xylem (Graniti and Perrotta 1988).

(Fig. 1)



Phoma tracheiphila. A. Pycnidium, vertical section. B. Ostiolar region of pycnidium, surface view. C. Part of pycnidial wall and conidiogenous cells producing conidia, vertical section. D. Conidia. E. Conidia being formed from hyphae (From Punithalingam and Holliday 1973).

On potato dextrose agar at 20-25 °C, conidia freely produced on hyphae. Conidiophores semimacronematous, mononematous, simple, septate, sometimes branched. Conidiogenous cells monophialidic, integrated, ampulliform to lageniform, determinate with well-defined collarettes. Conidia aggregated in slimy heads, semi-endogenous, simple, unicellular, straight, ends rounded, bi-guttulate, 2-2.5 (4.5-6.5 according to Sutton 1980) x 1-1.5 µm (Punithalingam and Holliday 1973, Z. Solel, pers. comm.).

Culture media and environmental conditions have a significant effect on colony characters and pigment production (Graniti 1969, Goidanich and Ruggieri 1953). Older cultures often lose their ability to produce pycnidia but still produce hyphal conidia (Graniti 1969, Solel pers. comm). Pigments are excreted on the hyphal surface as reddish-brown crystalline aggregates. The anthraquinonic pigments helminthosporin and its oxidized derivative cynodontin are produced by the fungus, as well as an additional yellow pigment (Graniti 1969).

Two types of variants may develop in culture (see Graniti 1969): nonchromogenic strains that lack red pigmentation but with brown aerial hyphae, and chromogenic strains with variable ability to produce red pigments but with hyaline hyphae. The latter strain irreversibly loses its capacity to produce pycnidia, brown hyphae, and sometimes hyphal conidia.

Phoma tracheiphila has no known teleomorph (perfect or sexual state).

A similar vascular pathogen, Phoma tracheiphila f. sp. chrysanthemi Baker et al., causes a mild chrysanthemum decline in California (Baker et al. 1985). That fungus is morphologically identical to the fungus causing mal secco but differs in host range. Inoculation tests on rough lemon and sour orange with this chrysanthemum parasite were unsuccessful (Baker et al. 1985).

Nachmias et al. (1979) reported the use of enzyme-linked immunosorbent assay (ELISA) for early detection of P. tracheiphila and diagnosis of mal secco disease.

Characteristic Damage

Mal secco is a tracheomycotic disease causing wilt and dieback of citrus trees. The pathogen causes a characteristic leaf veinal chlorosis (Fig. 2), followed by shedding of the leaves and eventual dieback of the twigs and branches (Fig. 3). Following death of the woody tissue, the fungus invades the bark and forms pycnidia under the epidermis, which becomes an ashy or lead gray color.

(Figs. 2-3)

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Phoma tracheiphila damage. 2. Veinal chlorosis of sour orange leaves. 3. Dying and dead branches on lemon tree (Courtesy C. N. Roistacher).

Symptom development depends on the part of the tree initially infected. Most infections occur through wounds, often in the canopy. Canopy infection spreads quickly upward resulting in leaf and branch collapse; the fungus eventually moves downward into the main branches and the trunk. Infection of a main limb results in rapid wilt and dieback of the portion of the tree above that limb. In Sicily, the "mal fulminante" form of the disease, associated with either root or trunk infections, leads to a sudden wilting of branches or of the whole tree (Graniti and Perrotta 1988, Z. Solel, pers. comm.). The "mal nero" form (Cutuli 1972) is associated with browning of the heartwood due to deep infections in trees. Initially asymptomatic, wilting and death occur abruptly when the fungus reaches functional xylem (Graniti and Perrotta 1988). A common host response to infection is production of sprouts from the base of affected branches or stems, and from the rootstock (Graniti and Perrotta 1988).

Vascular tissue of newly infected wood before wilt or death of the twig or branch has a characteristic orange-yellow or pink-salmon discoloration (Fig. 4), which later changes to a darker orange and eventually to brown or black as the disease progresses. The orangish discoloration is the most diagnostic

(Fig. 4)

Upper: pointer indicates typical discoloration of vascular tissue as seen in diagonally-cut mal secco affected branch. Lower: healthy cut branch (Courtesy C. N. Roistacher).

field character for mal secco, whereas other symptoms such as branch dieback and gray bark can be caused by other organisms also (Chitzanidis 1982).

Fruit on severely diseased branches may become infected and show some vascular discoloration (Ruggieri 1940, Savastano and Fawcett 1930); infected fruits are of poor quality and generally fall from the tree.

Detection Notes

Movement of infected plant parts of Citrus spp. and other rutaceous hosts could introduce the pathogen into new areas. Infected propagative material would be the most likely means of spread. This material could easily serve as a source of inoculum due to conidium production in and on infected plant parts. The likelihood of infected fruit serving as a source of disease spread is very low; they are of such poor quality that they are unlikely to be marketed or exported. Leaves become infected but are chlorotic and dry as a result so are unlikely to be moved commercially for consumption. Seed transmission of P. tracheiphila is currently under study (A. Graniti, pers. comm.).

To minimize the risk of introducing this citrus disease, Title 7 of the Code of Federal Regulations, Part 319.19 prohibits the entry into the United States of plants or plant parts (including cut flowers but not fruit or seeds) of members of the Rutaceae subfamilies Aurantioideae, Rutoideae, and Toddalioideae from all foreign localities. Entry of citrus fruit from all foreign areas including localities infested with mal secco is subject to the provisions of CFR 319.56. Citrus seed enters under CFR 319.37. Propagative material for scientific purposes enters under Departmental permit subject to stringent entry conditions including testing.

To detect mal secco in the field, (1) look for leaf and shoot chlorosis, wilt, and defoliation, followed by dieback of twigs and branches. Upper canopy infections spread slowly, but rapid wilting and death of portions of a tree may occur as a result of infection of lower portions of the tree or at the base of large branches.

(2) On live twigs and branches, make diagonal cuts and look for the diagnostic orange-yellow to pink-salmon discoloration that develops in newly infected twigs and branches (Fig. 4). Pigmentation can be accentuated by wetting the cut surface of the wood with a 1 percent solution of potassium hydroxide (Z. Solel, pers. comm.). The discoloration becomes brownish or black in later stages of the disease.

(3) On dead twigs and branches, look for bark that has become gray. Examine microscopically for embedded pycnidia containing minute, colorless conidia.

When submitting material for identification or confirmation of P. tracheiphila, discolored twigs and branches or bark with pycnidia will be most useful for identification purposes. Suspect plant material should be dried, labeled, and placed in sealed double containers.

Biology

Inoculum in the form of conidia is waterborne from the infected host tissue on or in which the fungus has sporulated (Punithalingam and Holliday 1973), including branches, leaves, and, to a lesser extent, fruits on the ground (Holliday 1980). Conidia or small pieces of infected plant material are easily removed and spread by rain and wind. Dissemination by contaminated birds and insects has been reported (Graniti and Perrotta 1988). The fungus enters the plant mainly through injuries to the leaves, branches, trunk, and roots. Entry through stomata has not been proven (Zucker and Catara 1985). The fungus then moves within the xylem mainly via the transpiration stream but also by mycelial growth in the vessels. The mycelium produces diffusable toxins that cause local necrosis within the plant (Perrotta et al. 1980). Young tissue is particularly susceptible to infection, for example, sprouts and suckers (Graniti and Perrotta 1988). Fungal penetration can occur in the zone where fruits are attached to the peduncle (Baldacci et al. 1950, Holliday 1980). Such infection causes rapid yellowing and premature fruit fall.

Conditions conducive to disease development include high relative humidity and temperatures between 14 and 28 °C, with 20-25 °C optimum for pathogen growth, conidium germination, and symptom development (Graniti and Perrotta 1988, Salerno 1964). Temperatures above 30 °C are fungistatic to the fungus in vivo. Optimal temperatures for pycnidium development in vitro range from 10 to 15 °C. Inoculated sour orange seedlings developed more pycnidia at 10.5 °C than at 20-22 °C (Graniti and Perrotta 1988, Salerno 1964). The disease rapidly spreads during autumn through early spring; spread in the host ceases at high summer temperatures (Punithalingam and Holliday 1973). Infection and transmission occur from November through February in Sicily (Goidanich 1964) and mid-November through mid-April in Israel, coinciding with the rainy season (Solel 1976). Conidial germinability is reduced during hot, dry periods (Grasso and Perrotta 1980). Symptoms begin to appear in early spring (Graniti and Perrotta 1988).

Development of races of P. tracheiphila has been postulated (see Salerno and Cutuli 1976); however Luisi et al. (1979) found no apparent specialization of the fungus. Some variability in virulence is reported (Salerno and Perrotta 1966).

Control

A combination of cultural practices, use of resistant cultivars, and to a lesser extent chemical treatments, is used to manage and control mal secco (Salerno and Cutuli 1979). However both cultural practices and chemical treatments are expensive, sometimes prohibitively. Strict quarantine regulations are in effect in many countries to prevent the introduction of P. tracheiphila into disease-free areas (Graniti and Perrotta 1988).

Cutting and burning infected twigs and branches are fundamental to the control of mal secco because they reduce the potential amount of fungal inoculum. Symptomatic portions of the tree to below the internally discolored areas should be removed by pruning throughout the season. Removal of stumps of infected trees is also recommended to prevent pycnidium development on sprouts. Light pruning "hardens" the trees making them less susceptible to infection. Injuries due to climatic factors such as wind, hail, or frost should be averted, for example, by using windbreaks and plastic netting. Disease progress can be slowed by applying low levels of nitrogen and adding phosphorus and potassium to the fertilizer. Cultivation of soil in late autumn and winter, especially in Sicily, increases the chance for root infection due to injury and should be avoided. Prolonged nontillage can also be detrimental apparently because roots develop near the soil surface, thereby increasing chances of injury and infection.

Protective and systemic fungicides have been used to aid in the control of mal secco (Luisi et al. 1976, Salerno and Cartia 1965, 1967, Salerno and Somma 1971, Solel 1979, Solel et al. 1972, Somma et al. 1974). Chemicals must be reapplied during the infection season, especially when new growth or wounding has occurred (Graniti and Perrotta 1988, Z. Solel, pers. comm.). Development of benomyl-resistant fungal strains is a problem (Salerno and Cutuli 1979).

Host resistance, in conjunction with cultural practices, is thought to be the most promising and desirable means of control (Luisi et al. 1980), especially where the disease is endemic. Any practice that enhances vigor of the tree, such as rootstock or fertilization, seems to increase its susceptibility (Z. Solel, pers. comm.). Nucellar lines are often much more

susceptible than old lines of the studied cultivars (Perrotta and Tribulato 1979). Resistant rootstocks are recommended (see HOST section for additional information).

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